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Phytoaccumulation of Heavy Metals in a Waste Engine Oil-contaminated Soil by *Aspillia africana* After Exposure to Hydroxyl Amine Hydrochloride Pre-treatment

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Authors' contributions

This work was carried out in collaboration between both authors. Author BI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author CCC managed the literature searches, analyses of the study performed the spectroscopy analysis and author BI managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

This study employed the use of $NH_2OH.HCl$ in the pre-treatment of *Aspilia africana* for the phytoremediation of waste engine oil-contaminated soil for a period of three months. 8 kg of soil was obtained, weighed and polluted at 5% W/W of waste engine oil which was replicated in 18 buckets and left for a period of one month to attenuate naturally before sowing with stems of *Aspilia africana* which were treated with four different concentrations of $NH_2OH.HCl$ (0.03125%, 0.0625%, 0.1250% and 0.2500% w/v) by soaking in the solution for one hour after which the planted stems were exposed for another two months with vegetative and physico-chemical parameters measured.

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The control was treated in distilled water (i.e. 0% w/v). Concentrations of heavy metals as well as Polyaromatic hydrocarbon in the contaminated soil were high after contamination and thus would normally be expected to impact the nutrients in the soil responsible for plant growth; with Cu present at a concentration of 26.24 mg/kg at 1 month after contamination (MAC) as compared to their none availability in the soil before contamination. As the plants matured with increase in time however, heavy metal concentrations in the soil decreased to the barest minimum in the soil with Cu (26.24 mg/kg at 1 MAC) been remediated to 0.40 mg/kg at 3 MAC using 0.0312% concentration of $NH_2OH.HCl$ treated *Aspilia africana*. It was observed that a considerable amount of Heavy metal concentrations in the contaminated soil were gotten rid of in the soil within the three months period that the experiment was carried out. Phytoremediation technology had worked for the degradation of contaminants in the soil and thus rendered the soil good for other useful purposes including agriculture and also to be kept for future reclamation activities. It is expected that further studies on this method of remediation will expose us to greatly appreciate nature's gift of cleansing our environment for mankind.

Keywords: Aspilia africana; bioremediation; hydroxyl amine hydrochloride; mutation breeding; phytoaccumulation; waste engine oil.

1. INTRODUCTION

1.1 Background

Petroleum hydrocarbon contamination of the environment has over the years posed a major global environmental threat to the health of humans found in such environment as well as the vegetations and wildlife. In spite of the problems associated with petroleum exploitation, particularly that which has to do with soil contamination, environmental managers can decide from a category of approaches to remediate petroleum-contaminated soil. These approaches range from thorough engineering techniques to natural attenuation which involves a 'hands-off' approach relying entirely on natural processes to remediate sites with no human involvement [1]. The on-site or *in situ* applications of plants and plant-related microorganisms to remove, contain, or stabilize toxic contaminants in soil or groundwater, otherwise referred to as phytoremediation, also lend a very important strategy to contaminant remediation [2].

One of the mechanisms of phytoremediation is phytoextraction; the accumulation of toxic cations into plant parts from the roots. This procedure uses plants to absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass such as shoots, leaves, etc. Different plants possess the capacity to tolerate and accumulate metals such as selenium, copper, cadmium and zinc [3,4,5]. It is also important to note that plants, with their associated rhizospheric microorganisms, have been found to enhance the removal of petroleum hydrocarbons from contaminated soils [6,7]. Some plant species can grow in contaminated plots and thus successfully resist the soil contamination by petroleum hydrocarbons. They do this by not only adapting to the adverse conditions of the soil; they also direct their metabolism in such a way that the plant tolerance level of contamination is as high as possible [8]. One way to identify prospective plants for phytoremediation is to take note of the species that naturally inhabit contaminated soils [8].

Studies have shown that exposure of plants to certain mutagenic agents have enhanced their growth and development, which is sine qua non to successful phytoremediation activities of the plant. Phytoextraction, for example relies on enhanced plant biomass, highly developed rooting system as well as enhanced foliage formation; these properties have been reportedly enhanced by exposure of the plants to mutagenic agents like sodium azide and hydroxylamine hydrochloride [9,10,11,12,13]. This research work, therefore, seeks to assess the effectiveness of phytoremediation technology of the hydrocarbon-contaminated soil with the use of local *Aspillia africana* which is a tropical shrub widely grown in Nigeria as an alternative to the un-environmentally friendly processes being practiced in the past. A number of mutagenic agents has been reported to enhance above-ground and below-ground plant parameters [11]. These two groups of parameters which include; foliage and root development are key to successful phytoremediation strategies.

The study aims to harness these characteristics by pre-treatment of *Aspillia africana* stems in NH_2-OH.HCl. [14,15] reported the preponderance of Asteraceae plants in oil-contaminated areas as well as in metal-contaminated soils in Edo / Delta States of Nigeria; hence the choice of *Aspillia africana*. The findings will be useful in the remediation of contaminated soils in the oil-producing regions of Nigeria and other oil contamination related problems around the globe in the near future.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Materials for the Experiment

Prior to the study, stem cuttings were obtained from healthy *Aspillia africana* plant stands on an abandoned experimental farm beside the University Main Library. Soil used in the present study was collected from an area measuring 20 m x 25 m marked on the same area where stem cuttings were obtained. Top soil (0 - 10 cm) was collected randomly from the marked plot and sun-dried to constant weight. Ten (10) kg of soil was measured into plastic buckets with five 8-mm diameter perforations underneath. Waste engine oil (WEO) was obtained as pooled sample from petrol-engine vehicles in an auto-mechanic workshop in Ikpoba Hill, Benin City. Prior to exogenous application of waste engine oil (WEO), precontamination concentrations of heavy metals as well as other physiochemical parameters of the unpolluted soils were determined.

2.2 Soil Contamination Using Varying Levels of WEO in Each Bucket

Soils in each bucket were thoroughly mixed with WEO on weight basis, to give a constant 4% w/w concentration. Soils were then allowed to attenuate for one month, after which stem cuttings were pretreated and then sown.

2.3 Preparation of Mutagenic Solution

Hydroxylamine hydrochloride (NH_2OH.HCl) solution was used in the present study as mutagenic agent. The solution was prepared on weight per volume basis by dissolving 0.3125g NH_2OH.HCl salt in 100 litres of distilled water, pH 7.0 (0.3125% w/v NH_2OH.HCl in solution, Mshembula et al., 2012). Three other levels were prepared on weight per volume basis, viz: 0.0625%, 0.125%, and 0.25%. The control was distilled water at pH 7.0 (0% w/v).

2.4 Pre-treatment of Stems Cuttings with Sodium Azide

The stem cuttings of relatively uniform length (30 cm) and sizes were fully submerged in NH_2OH.HCl solution for one hour. They were then removed and washed in running water to remove excess chemicals and exudates, and taken immediately to field where they were sown directly into the control and polluted soils.

Three (3) stem cuttings of equal size (2 cm thickness) and length (30 cm) were planted in each bucket, separated from each other in a triangular manner. The soil in buckets was weeded as weeds appeared. Although the plants were exposed to the prevailing weather condition, water requirements by the crop were supplemented during very dry days by addition of 200 ml distilled water applied per bucket before sunset. The plants were monitored for 2 additional months, after which the experiment was terminated.

2.5 Soil Physicochemical Analyses

Soils were dried at ambient temperature (22-25ºC), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of selected soil physicochemical properties and the heavy metal fractions.

Ten (10) g of soil was weighed into a 250 ml plastic bottle. 100 ml of 0.1 m HCI was added, stoppered, and then shaken for 30 minutes. The mixture was filtered through Whatman filter paper No.42 and then Cr, V, Pb, Ni, Mn, and Cu were determined in the filtrate by Atomic Absorption Spectrometer [16,17]. Determination of TAH and PAH contents of soil were according to methods described by [18].

2.6 Efficiency of Heavy Metal Removal

This was presented as a percentage and calculated as:

$$\frac{\text{SHM at 3MAC}}{\text{SHM at 1 MAC}} \times \frac{100}{1}$$

Where SHM = Sum of concentration of all assayed heavy metals in the soil at a given time and treatment. MAC = months after contamination.

2.7 Phytoaccumulation Factor (PF)

This was calculated as:

$$\frac{\text{Concentration of heavy metal in plant part}}{\text{Residual concentration of heavy metal in soil}}$$

When PF>1, significant accumulation. This parameter was only considered in the leaves of the plants.

Results of the study were captured as means of 5 replications. The means were statically separated using the Duncan Multiple Range Test from the SPSS-16 statistical software package.

3. RESULTS

The physical and chemical properties of soil and waste engine oil (WEO) used for the experiment has been presented on Table 1.

Cu with an initial concentration of 26.24 mg/kg at 1MAC, prior to sowing reduced to 0.40 mg/kg in the soil sown with 0.0312% w/v $NH_2OH.HCl$-treated plants (Table 2). Cu concentration in the soil without plant presence was 16.21 mg/kg. There was total removal of Mn and V in soil occasioned by sowing $NH_2OH.HCl$-treated plants. The efficiency of heavy metal removal by the plants at 3MAC/2MAS ranged from 32.11 – 36.66 %, compared to 23.77% in the soil without plant presence.

At 1 MAP, PAH contents of soil ranged from 10.05 162.95 mg/kg prior to sowing of treated and untreated stem cuttings of *A. Africana* (Table 3). Two months after sowing the plants on oil-contaminated soil, total PAH of soil was 441.24 mg/kg, indicating a 53.57% efficiency of PAH removal. With the presence of plants in the soil, total PAH contents ranged from 155.36 - 329.20 mg/kg respectively. Obviously, the presence of plants facilitated the removal of PAH from soil. Comparatively, mutagen-treated plants possessed significantly (p>0.05) higher efficiencies of PAH remediations. The lowest PAH contents were obtained in the soil that was sown 0.125% w/v $NH_2OH.HCl$ – treated plants. Acenaphthylene and acenaphthene were below detectable limits in all soils that contained treated plants.

Table 1. Physical and chemical properties of soil and waste engine oil (WEO)

Parameters	Soil	WEO
pH	6.23	5.85
Electrical conductivity (µs/cm)	298	NA
Total org. matter (%)	0.56	NA
Total nitrogen (%)	0.14	NA
Exchangeable acidity (meq/100 g soil)	0.21	NA
K (meq/100 g soil)	1.34	NA
Ca (meq/100 g soil)	10.98	NA
Mg (meq/100 g soil)	9.93	NA
P (mg/kg)	142.31	NA
Clay (%)	9.50	NA
Silt (%)	12.98	NA
Sand (%)	77.52	NA
Cu (mg/kg)	BDL	52.21
Mn (mg/kg)	BDL	12.65
Ni (mg/kg)	BDL	69.68
V (mg/kg)	BDL	5.21
Cr (mg/kg)	0.03	86.32
Cd (mg/kg)	BDL	1.25
Zn (mg/kg)	0.01	102.35
Pb (mg/kg)	BDL	19.65
Fe (mg/kg)	986.35	3069.45

BDL= Below Detectable Limit (0.0001 mg/kg); NA= Not available

The heavy metal accumulation in plant leaves at 2 months after sowing in a 1 month old naturally attenuated waste engine oil-contaminated soil have been presented on Table 4. Cu, Cr, Zn, and Fe were successfully accumulated in the leaf of the plant with Fe being the most accumulated (105.84 – 362.92 mg/kg) while Pb was the least accumulated (0.01 – 0.03 mg/kg). These have been further demonstrated by their respective phytoaccumulation factors (PF) (Table 5). PF gives insight into the efficiency of the phytoremediation capability of the plant. When PF = 0, plant is not an accumulator. However PF < 1 signifies minimal plant accumulation, while PF > 1 signifies a hyper-accumulation. Mn, Ni, V, Cd and Pb were not accumulated in plant leaves. However, it was observed that PF for Cu increased with the increasing concentrations of mutagenic treatment.

Table 2. Heavy metal concentration of oil-contaminated soil at 3 months after contamination

Heavy metals	1 MAC (before sowing)	3 MAC/2 MAS					
		Concentration of $NH_2OH.HCl$					
		No plant	0%	0.0312%	0.0625%	0.1250%	0.2500%
Cu	26.24[a]	16.21[b]	6.35[c]	0.40[c]	3.00[c]	4.25[cd]	3.65[c]
Mn	4.30[a]	0.02[b]	BDL[c]	BDL[c]	BDL[c]	0.01[b]	0.01[b]
Ni	35.11[a]	22.16[b]	12.70[c]	10.25[c]	13.20[c]	9.23[c]	11.52[c]
V	1.03[a]	0.62[a]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]
Cr	51.33[a]	33.32[b]	10.70[d]	13.50[d]	18.20[cd]	26.7[bc]	28.32[bc]
Cd	0.02[a]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]	BDL[b]
Zn	71.32[a]	63.32[ab]	41.04[c]	3.45[e]	2.46[e]	59.24[bc]	19.57[d]
Pb	8.85[a]	5.23[b]	0.06[c]	0.10[c]	0.29[c]	0.92[c]	1.23[c]
Fe	2601.25[a]	1993.21[b]	1702.32[b]	1765.21[b]	1804.94[b]	1800.21[b]	1771.59[b]
Efficiency (%)	-	23.77[c]	36.66[a]	35.96[a]	34.20[ab]	32.11[b]	34.42[ab]

BDL= Below Detectable Limit (0.0001 mg/kg). Means on the same rows with similar alphabetic superscripts do not differ significantly from each other (p<0.05). MAC months after contamination. MAS months after sowing. 0% w/v as treatment was water

Table 3. Polyaromatic hydrocarbon contents of soil at 3 months after pollution and 2 months after sowing treated and control stems of *Aspillia africana* in oil-polluted soil

Components	1 MAP	3 MAP/2MAS					
		No plant	Concentration of $NH_2OH.HCl$ used for pre-treatment (% w/v)				
			0	0.03125	0.0625	0.125	0.25
Naphthalene	26.32[a]	16.72[b]	15.32[b]	15.26[b]	17.29[b]	17.19[b]	15.25[b]
Acenaphthylene	10.05[a]	3.95[b]	BDL[c]	BDL[c]	BDL[c]	BDL[c]	BDL[c]
2-bromonaphthalene	29.63[a]	16.35[b]	12.65[b]	15.54[b]	17.52[b]	BDL[c]	BDL[c]
Acenaphthene	31.20[a]	16.33[b]	0.52[c]	BDL[c]	BDL[c]	BDL[c]	BDL[c]
Fluorene	39.68[a]	19.20[b]	3.68[c]	15.72[b]	17.66[b]	BDL[d]	15.59[b]
Phenanthrene	25.66[a]	17.52[b]	16.95[b]	15.76[b]	18.38[b]	18.63[b]	16.72[b]
Anthracene	27.98[a]	18.21[b]	15.95[b]	16.53[b]	18.51[b]	BDL[c]	16.46[b]
Fluoranthene	43.62[a]	16.97[b]	16.35[b]	BDL[C]	17.59[b]	BDL[c]	15.81[b]
Pyrene	36.09[a]	15.33[b]	16.32[b]	15.58[b]	17.50[b]	17.41[b]	15.69[b]
Benzo(a)anthracene	58.32[a]	38.62[b]	19.65[c]	18.42[c]	22.68[c]	BDL[d]	20.42[c]
Chrysene	119.05[a]	42.11[b]	33.28[bc]	19.49[d]	23.44[cd]	20.29[d]	17.96[d]
Benzo(b,j,k)fluoranthene	50.95[a]	34.91[b]	34.52[b]	29.41[bc]	21.74[cd]	19.25[d]	29.03[c]
Benzo(a)pyrene	162.95[a]	32.49[b]	46.98[b]	36.85[b]	24.39[b]	27.06[b]	38.95[b]
Indeno(1,2,3-cd)pyrene	120.89[a]	32.01[b]	24.25[b]	7.78[c]	1.87[c]	1.91[c]	0.83[c]
Dibenzo(a,h)anthracene	99.67[a]	57.41[b]	32.92[c]	12.64[d]	2.58[e]	5.75[e]	2.92[e]
Benzo(g,h,i)perylene	68.42[a]	63.21[a]	39.86[b]	23.99[bc]	25.35[bc]	27.87[bc]	22.03[c]
Total PAH	950.48[a]	441.34[b]	329.2[c]	242.99[cd]	246.51[cd]	155.36[e]	227.66[d]
Efficiency (%)	-	53.57[d]	65.36[c]	74.48[b]	74.06[b]	83.65[a]	76.04[ab]

BDL= Below Detectable Limit (0.0001 mg/kg). Means on the same rows with similar alphabetic superscripts do not differ significantly from each other (p<0.05). MAC months after contamination. MAS months after sowing. 0% w/v as treatment was water

Condition of *Aspillia africana* sown in a 1 month old naturally attenuated waste engine oil-contaminated soil at 2 months after sowing has been presented in Table 6. Assessed plant parameters were enhanced in the clean soil. Length of sprouted branches of NH2OH.HCl-treated plants increased from 12.31 – 37.31 cm in the oil-polluted soils. There was no significant difference in length of branches among plants pre-soaked in higher concentrations of NH2OH.HCl solution, compared to the control (32.02 cm). NH2OH.HCl-treated plants in oil-polluted soils showed broader leaves (leaf area, 29.00 – 30.14 cm^2) compared to the control (leaf area, 26.88 cm^2).

Table 4. Concentration of heavy metal in plant leaves of *Aspilia africana* at 2 MAS in a 1 month old naturally attenuated waste engine oil-contaminated soil

Heavy metals	Concentration of $NH_2OH.HCl$ (% w/v)				
	0	**0.0312**	**0.0625**	**0.125**	**0.250**
Cu	9.05^{a}	5.52^{b}	5.20^{b}	6.52^{ab}	4.221^{b}
Mn	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}
Ni	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}
V	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}
Cr	14.20^{a}	12.94^{ab}	11.94^{ab}	10.84^{b}	11.92^{ab}
Cd	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}	BDL^{a}
Zn	40.18^{a}	16.46^{b}	12.38^{b}	11.25^{b}	12.89^{b}
Pb	BDL^{b}	BDL^{b}	0.01^{a}	0.02^{a}	0.03^{a}
Fe	362.92^{a}	301.51^{a}	298.96^{a}	105.84^{b}	135.15^{b}

BDL= Below Detectable Limit (0.0001 mg/kg). Means on the same rows with similar alphabetic superscripts do not differ significantly from each other (p<0.05). MAC months after contamination. MAS months after sowing. 0% w/v as treatment was water

Table 5. Phytoaccumulation factor (PF) of *Aspillia africana* in relation to soil metal concentration at 2 MAS in a 1 month old naturally attenuated waste engine oil-contaminated soil

Heavy metals	Concentration of $NH_2OH.HCl$ used for pre-treatment (% w/v)				
	0	**0.0312**	**0.0625**	**0.125**	**0.250**
Cu	0.35^{c}	0.34^{c}	0.82^{b}	2.18^{a}	1.16^{b}
Mn	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Ni	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
V	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Cr	0.28^{b}	0.39^{b}	0.66^{a}	0.41^{ab}	0.42^{ab}
Cd	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Zn	0.56^{a}	0.26^{b}	0.30^{b}	0.19^{c}	0.66^{a}
Pb	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Fe	0.14^{a}	0.15^{a}	0.18^{a}	0.06^{a}	0.08^{a}

PF > 1 indicates significant hyperaccumulation. BDL= Below Detectable Limit (0.0001 mg/kg). Means on the same rows with similar alphabetic superscripts do not differ significantly from each other (p<0.05). MAC months after contamination. MAS months after sowing. 0% w/v as treatment was water

Table 6. Condition of *Aspillia africana* sown in a 1 month old naturally attenuated waste engine oil-contaminated soil at 2 months after sowing

Plant parameters	Concentration of $NH_2OH.HCl$ (% w/v)					
	0	**0**	**0.0312**	**0.0625**	**0.125**	**0.250**
	Unpolluted soil	**Oil-polluted soil**				
Length of sprouted branches (cm)	32.02^{a}	12.31^{b}	15.02^{b}	15.13^{b}	37.31^{a}	35.43^{a}
No. of sprouted branches	27.23^{a}	13.41^{b}	12.32^{b}	11.14^{b}	14.95^{b}	12.83^{b}
No. of leafs/plant	257.32^{a}	108.34^{b}	104.74^{b}	95.14^{b}	105.04^{b}	104.32^{b}
Leaf area (cm^2)	26.88^{ab}	21.00^{c}	24.91^{bc}	29.00^{a}	30.19^{a}	24.94^{bc}

Means on the same rows with similar alphabetic superscripts do not differ significantly from each other (p<0.05). MAC months after contamination. MAS months after sowing. 0% w/v as treatment was water

4. DISCUSSION

4.1 Effects of Remediation on Heavy Metal Contents of WEO-Polluted Soil

Petroleum hydrocarbon contamination of the environment poses a major global environmental threat to the health of humans, vegetation and wildlife. The remediative measures carried out on presence of heavy metals in soil to plants come in different forms. Plants may absorb, concentrate, and precipitate toxic metals from contaminated soils into the above ground biomass such as shoots, leaves, fruits, etc. [19].

Plants may also employ its roots to limit contaminant mobility and its bioavailability in the soil. This is done so as to; decrease the amount of water percolating through the soil matrix which may result in the formation of a hazardous leachate, act as a barrier to prevent direct contact with the contaminated soil, and prevent soil erosion and the distribution of the toxic metal to other areas [19]. Plants may take up contaminants from the soil, degrading them into volatile forms and transpiring them into the atmosphere or may choose to absorb contaminants from polluted aqueous sources in their roots.

Although plant resistance to these environmental pollutants has been demonstrated in previous studies, such as studies by [20] in *Cynodon dactylon,* [21] in *Solanum melongena* and *S. incarnum,* [22] in *Glycine max* and *Phaseolus vulgaris,* [23] in *Zea mays,* and [24] in *Ricinus communis,* it is however noted that the plant employed in this present study which is *Aspillia africana* has never been explored by previous researchers for its phytoremediative potentials but rather its medicinal or curative capabilities. However, other plants of the Asteraceae family have been researched upon for their remediative potentials. Such studies include studies by [25] in *Acanthospermum hispidum,* and [26] in *Chromolyna odorata*.

Phytoextraction as a mechanism of remediation whereby remediative plants remove and isolate heavy metal contamination from soil without causing destruction to the structure and physico-chemical parameters of the soil is actually the most suitable method of phytoremediation. Most often, it is referred to as phytoaccumulation. As the plant absorbs or concentrates toxic metals and radionuclide from contaminated soils into the biomass, it is best suited for the remediation of diffusely polluted areas, where pollutants occur only at relatively low concentrations and superficially.

An initial increase in heavy metal concentration in soil was recorded in the present study upon introduction of WEO at 1 MAC. From the first to the third month after soil contamination with WEO, there were gradual reductions in heavy metal composition of the polluted soil. Compared to heavy metal concentration (Fe, Mn, Cu, Zn, Cr, Cd, Pb, Ni, V and THC) at 1 MAC, there was significant mean reduction of heavy metals at 3 MAC/2 MAS across the various concentrations of $NH_2OH.HCl$ treated *A. africana* employed. There was reduction in Cu content from 26.24 mg/kg at 1 MAC to 16.21 mg/kg in NP (soil with No Plant) and 0.04 mg/kg in soil with 0.0312% $NH_2OH.HCl$ treated *A. africana* at 3 MAC/2 MAS. This indicates that the latter was most effective in the reduction of Cu from the polluted soil. Mn was reduced from 4.3 mg/kg at 1 MAC to 0.01 mg/kg in soil with 0.0125% and 0.2500% $NH_2OH.HCl$ treated *A. africana* and also there was a total eradication of Mn (0.0 mg/kg) in the soil with untreated plant, 0.0312% and 0.0625% $NH_2OH.HCl$ treated *A. africana* at 3 MAC/2 MAS. Fe was reduced from 2601.25 mg/kg at 1 MAC to 1804.94 mg/kg in soil with 0.0625% $NH_2OH.HCl$ treated *A. africana* and 1702.32 mg/kg in water-treated *A. africana* at 3 MAC/2 MAS.

4.2 Absorption of Contaminants by Leaves of *Aspilia africana*

The plant leaves at 2 months after sowing was able to successfully accumulate certain amount of heavy metals. Cu, Cr, Zn, and Fe were successfully accumulated in the leaf of the plant with Fe being the most accumulated while Pb was the least accumulated. Fe which was initially present in the unpolluted soil at a concentration of 986.35 mg/kg and present in the WEO at 3069.45 mg/kg was accumulated in the leaf of the untreated *Aspilia africana* plant at a concentration of 362.92 mg/kg while 135.15 mg/kg was accumulated in the leaf of *Aspilia africana* treated with 0.2500% $NH_2OH.HCl$ concentration at 2MAS. This shows that the greatest amount of phytoextraction of Fe occurred in the untreated plant. Pb being the least accumulated had an accumulation of 0.009 mg/kg using 0.0625% Concentration of $NH_2OH.HCl$ and 0.031 mg/kg using 0.2500% Concentration of $NH_2OH.HCl$. However, Mn, Ni, V and Cd were not accumulated at all despite their initial presence in the polluted WEO at varying concentrations of Mn (12.65 mg/kg), Ni (69.68 mg/kg), V (5.21 mg/kg) and Cd (1.25 mg/kg). It can however be noted that the above listed un-accumulated metals were initially not available in the pre-contaminated soil.

Phytoaccumulation factor gives insight into the efficiency of the phytoremediation capability of the plant. PF = 0 indicates that the plant is not an accumulator, PF < 1 shows plant accumulated but not significant while a PF > 1 indicates that the plant is a hyper-accumulator. At 2 MAS, Mn, Ni, V, Cd and Pb each possess a PF of zero across the various concentrations of $NH_2OH.HCl$ employed which is an indication that the plant was not an accumulator of these heavy metals.

However, it was observed that the phytoaccumulation factor for Cu increased with the increase in concentration from 0.34 mg/kg (0.0312%), 0.82 mg/kg (0.0625%), till its peak was attained at 2.18mg/kg using 0.1250% concentration and a slight decrease afterwards to 1.16 mg/kg using 0.2500% concentration of $NH_2OH.HCl$. It could be observed also that while the control plant and the plant treated with 0.0312% and 0.0625% concentration of $NH_2OH.HCl$ could be said to have accumulated the heavy metal though its accumulation was insignificance, same could not be said for the plant treated with 0.1250% and 0.2500% as they were both regarded as hyper-accumulators. Zn equally enjoyed an increase in the PF with increase the concentrations of $NH_2OH.HCl$ employed starting from 0.26 mg/kg using 0.0312% concentration, 0.30 mg/kg using 0.0625% concentration before attaining its peak at 0.66 mg/kg using 0.2500% $NH_2OH.HCl$ treated plant although there had been a slight reduction to 0.19 mg/kg using 0.1250% concentration. It was observed that the plant showed insignificant accumulation for Cr, Zn, and Fe across the various concentrations of mutagen employed as their phytoaccumulation factor were less than 1.

4.3 Impact of $NH_2OH.HCl$ and WEO-Polluted Soil on Growth and Yield Parameters of *Aspilia africana*

Hydrocarbon changes soil properties because hydrophobic soil behaviour can lead to decrease in water availability and the rate at which nutrients are accessible [27,28]. [8] in *Helianthus anuus* indicated that the making of drought conditions under oil-contamination stress has effect on some growth parameters. This drought stress can lead to reduction in root and leaf cells water potential to a lower level needed for cell elongation. Furthermore, inhibition of plant growth can be caused by toxic compounds of petroleum hydrocarbons [28,29].

In this present study, the length of sprouted branches of *Aspillia africana* sown in a 1 month old naturally attenuated waste engine oil-contaminated soil showed that at 1 week after sowing, the stem treated using 0.6250% Concentration of $NH_2OH.HCl$ had a length of 2.5 cm and was the most sprouted at this period of growth. It might be that the concentration employed had a positive impact on the stem's ability to overcome the lag phase of germination. At week 4 however it was observed that there was reduction in the growth rate of the stem treated with 0.0625% Concentration of $NH_2OH.HCl$ (12.0 cm) which was initially the most sprouted at the first week of sowing when compared to the 25 cm length recorded for the stem treated with 0.250% Concentration of $NH_2OH.HCl$ which is among the most sprouted at this period of growth. At the final week of sowing (8^{th} week), branches of the stems treated with 0.1250% and 0.2500% concentrations of $NH_2OH.HCl$ were among the most sprouted with length of 37 cm and 35 cm respectively which might be an indication that at this period, the stems were fully adapted to their new found surroundings.

The number of sprouted branches of *Aspillia africana* sown in a 1 month old naturally attenuated waste engine oil-contaminated soil when examined at the final week of sowing which is 2MAS (8^{th} week), branches of the control plant (0%) having a total number of 13 and the branches of stems treated with 0.2500% concentrations of $NH_2OH.HCl$ with a total number of 12 were among the most sprouted when compared to the branches of the stems treated with 0.1250% $NH_2OH.HCl$ which happened to produce the least number of sprouted branches with a total branch number of 3 at 2MAS (8^{th} week). The general fluctuations noticed in the number of sprouted branches across the various concentrations could be attributed to the effect of some biotic and abiotic factors that can result in wilting or necrosis of the already sprouted branches.

At the final week of sowing, a check on the number of leaves showed that stems treated with 0%, 0.0312%, 0.0625%, and 0.2500% concentrations of $NH_2OH.HCl$ were among the stems with the most sprouted number of leaves while the stem treated with 0.1250% $NH_2OH.HCl$ happened to produce the least number of leaves with a total leaf number of 35 at 2MAS (8^{th} week). It can also be noticed that there was a general fluctuation in the number of leaves across the various concentrations. Also, the general fluctuations noticed in the number of sprouted branches across the various concentrations could be attributed to the effect of some biotic and abiotic factors that can result in wilting or necrosis of the already sprouted leaves.

It was observed that the leaf area (cm^2) of *Aspillia africana* sown in a 1 month old naturally attenuated waste engine oil-contaminated soil recorded 1.80 cm^2 for the untreated stem of *A.africana* and the stem treated using 0.6250% Concentration of $NH_2OH.HCl$ had a leaf area of 2.56 cm^2 in the first week of sowing. At week 8 however, it was observed that the leaves of stems treated with 0.0312%, 0.0625%, 0.1250%, and 0.2500% concentrations of $NH_2OH.HCl$ were among the largest in terms of leaf area while the leaves of the control plant (0%) $NH_2OH.HCl$ happened to produce the leaves with the least area with a final leaf area of 9cm^2 at 2MAS (8th week). According to [8] in the study on Sunflower, the reduction of growth which must result in reduction of biomass is the first negative effect of petroleum hydrocarbons on plants. It was also reported that although decrease of some growth parameters such as root length and leaf area was not related to reduction of total biomass of plant under contaminated conditions, it seems a portion of the biomass was generated from accumulation of heavy crude oils in tissues by phytolignification.

5. CONCLUSION

It is a general knowledge that petroleum hydrocarbon contamination of the environment poses a major threat to both the natural and economic resources of the populace affected and hence phytoremediation offers a less expensive, eco-friendly, and unsophisticated means of rendering contaminants harmless. It is expected that further studies on this method of remediation will expose us to greatly appreciate nature's gift of cleansing our environment to mankind.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Frick CM, Farrell RE, Germida JJ. Assessment of phytoremediation as an *in situ* technique for cleaning oil-contaminated sites. Petroleum Technology Alliance of Canada; 1999.
2. Cunningham SD, Anderson TA, Schwab AP, Hsu FC. Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 1996;56:55-114.
3. Brown SL, Chaney RL, Angle JS, Baker AJM. Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soil. Journal of Environmental Quality. 1994;23: 1151-1157.
4. Banuelos GS, Ajwa HA, Mackey B, Wu L, Cook C, Akohoue S, Zambruzuski S. Evaluation of different plant species used for phytoremediation of high soil selenium. Journal of Environmental Quality. 1997;26: 639-646.
5. Ebbs SD, Lasat MM, Brady DJ, Cornish J, Gordon R, Kochian LV. Phytoextraction of cadmium and zinc from a contaminated soil. Journal of Environmental Quality. 1997;26:1424-1430.
6. Schwab AP, Banks MK, Arunachalam M. Biodegradation of polycyclic aromatic hydrocarbons in rhizosphere soil. Bioremediation of Recalcitrant Organics. Hinchee RE, Anderson DB, Hoeppel RE. Battelle Press: Columbus. 1995;23–29.
7. Ikhajiagbe B. Synergism in Bioremediation: Phytoassessment of waste engine oil polluted soils after amendment and bioaugmentation. LAP Lambert Academic Publishing, Köln, Germany. 2010;276.
8. Fatemeh GR, Khadijeh K, Ravanbakhsh S. Effects of petroleum hydrocarbons on growth, photosynthetic pigments and carbohydrate levels of sunflower. Journal of Food, Agriculture & Environment. 2012; 10(1):773-776.
9. Mensah JK, Akomeah PA. Mutagenic effects of hydroxylamine and streptomycin on the growth and seed yield of cowpea (*Vigna unguiculata* (L.) Walp). Legume Research. 1992;15(1):39-44.
10. Mensah JK, Obadoni BO, Akomeah PA, Ikhajiagbe B, Ajibolu Janet. Effects of sodium azide and colchicines treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). African Journal of Biotechnology. 2006;6(5): 534-538.
11. Mshembula BP, Mensah JK, Ikhajiagbe B. Comparative assessment of the mutagenic effects of sodium azide on some selected growth and yield parameters of five accessions of cowpea – Tvu-3615, Tvu-2521, Tvu-3541, Tvu-3485 and Tvu-3574. Archives of Applied Science Research. 2012;4(4):1682-1691.
12. Omoregie UE, Mensah JK, Ikhajiagbe B. Germination response of five varieties treated with sodium azide. Research Journal of Mutagenesis (published as online first); 2012. ISSN 1994-7917 / DOI: 10.3923/rjmutag.2012.

13. Ikhajiagbe B. Odigie, Ujomonigho E, Okoh B. Efenaide, Agho E. Esther. Effects of sodium azide on the survival, growth and yield performance of rice (*Oryza sativa*, FARO-57 variety) in a hydrocarbon-polluted soil. The International Journal of Biotechnology. 2013;2(1):28-41.
14. Anoliefo GO, Ikhajiagbe B, Okonokhua BO, Diafe FV. Ecotaxonomic distribution of plant species around auto mechanic workshops in Asaba and Benin City: Identification of oil tolerant species. African Journal of Biotechnology. 2006;5(19): 1757-1762.
15. Anoliefo GO, Ikhajiagbe B, Okonokhua BO, Edegbai BE, Obasuyi OC. Metal tolerant species distribution and richness in and aroud metal based industries: Possible candidates for phytoremediation. Africa Journal of Environmental Science and Technology. 2008;2(11):360-370.
16. Adeniyi AA. Determination of Cadmium, Copper, Iron, Lead, Manganese, and Zinc in water leaf (*Talinum triangulare*) in dumpsites. Environment International. 1996;259:259-262.
17. AOAC. Methods of Analysis. Association of official analytical chemists. Washington D. C. 2005;326.
18. Dean JR, Xiong G. Extraction of organic pollutants from environmental matrices: selection of extraction technique. Trends in Analytical Chemistry. 2000;19(9):553–564.
19. Henry JR. An overview of the phytoremediation of lead and mercury. U.S. Environmental Protection Agency; 2000.
20. Wong MH, Chu LM. Yield and metal uptake of *Cynodon dactylon* (*Bermuda grass*) grown in refuse-compost-amended soil. Agriculture, Ecosystems and Environment. 1985;14(1-2):41-52.
21. Anoliefo GO, Edegbai BO. Effect of spent engine oil as a soil contaminant on the growth of two egg plant species, *Solanum melongen* L and *S. incanum*. Journal of Agriculture, Forestry and Fisheries. 2001; 1:21–25.
22. Dominguez-Rosado E, Pitchel J. Phytoremediation of soil-contaminated with used motor oil: II. Greenhouse studies. Environmental Engineering Studies. 2004; 21:169-180.
23. Ogboghodo IA, Iruafa EK, Osenwota IO, Chokor JU. An assessment of the effect of crude oil pollution on soil properties: Germination and growth of maize. Proceedings of the 27th Annual Conference of the Soil Science Society of Nigeria Nov 5th – 9th, University of Calabar, Nigeria; 2001.
24. Vwioko DE, Fashemi DS. Growth response of *Ricinus communis* L (Castor oil) in spent lubricating oil-contaminated soil. Journal of Applied Sciences and Environmental Management. 2005;9:73-79.
25. Ikhajiagbe B, Anoliefo GO. Natural attenuation of a 14-month-old waste engine oil polluted soil. Journal of Soil Science and Environmental Management. 2011;2(7):184-192.
26. Baah B. Phytoremediation of hydrocarbon contaminated soil - a case study at Newmont Ghana Gold Limited – Ahafo Kenyasi. Kwame Nkrumah University of Science and Technology. 2011;1-6.
27. Baker JM. The effect of oils on plants. Environment and Pollution. 1970;1:27-44.
28. Bossert L, Bartha R. Plant growth on soils with a history of oily sludge disposal. Soil Sci. 1985;140(1):75-77.
29. Reis JC. Environmental control in petroleum engineering. Gulf Publ., Houston, TX. 1996;274.

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